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A CHEMICAL STUDY OF WHEAT.

By

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A Chemical Study of Wheat.

This study and research work was started with the idea of trying to learn more about the chemical characteristics of the components of the wheat kernel. In the short time it has nôt been possible to study all the compounds. Also there is so little known about any of them that the study of one offers a very large field for research work. In this paper the work has been almost wholly limited to the study of gliadin, one of the important proteins of the wheat kernel.

The past literature on the subject is quite voluminous. A great many articles have been written in the various scientific publications but a great many are mere repetitions. Most of the literature comes from the various Experiment Stations located throughout the United States and Canada. It is interesting and valuable in that it deals with wheat grains under very different physical and climatic conditions. The influence of conditions and the difference in wheats produced under

them is of a great value to the men in charge of Experiment Station work. The work consists for the greater part in a large number of analysis of the wheat kernel. Then the physical characteristics are noted and the wheat milled on an experimental mill. After which each flour produced is baked. In this way they have attempted to derive some relations between the chemical constituents and the baking value of a flour produced from such a wheat. Each crop of wheat and also wheat in the same crop differs greatly, due to the soil, moisture and various climatic conditions. A soft Winter Kansas wheat differs greatly from a hard Minnesota wheat in physical and baking qualities although the percentage of the different constituents in the two wheats may be the same. This is perhaps a fairly exceptional case. On the whole the results obtained are very general in their application. Very little real chemical work, with the exception of the analysis, has been done in this line. By this is meant that the real chemical nature of the different constituents has not as yet been worked out.

In this line several English and German chemists have done admirable work. In this country, Osborne [1] & [2] and his associates have been pioneers in the work.

In the beginning it must be realized that the wheat kernel is a very complex combination. Just how the different constituents stand to one another and what their exact relations are has never been determined. One may be a development from the other, or they may all develop singly or they may all develop from one constituent formed first. The different compounds found are, carbohydrates, fibre, proteins, fat, mineral matter or ash, enzymes and bacteria.

The carbohydrates are principally starch and sugar, of these starch is the principal constituent. The starch is located in a larger per cent nearer the center of the kernel, being in the least amount next to the bran coat. The bran coat is the fibre of the wheat kernel.

The percentage of fat in wheat forms a small per cent of the whole wheat. The fat carries most of the coloring matter. It is acid in character and is easily acted on by oxidizing agents in very small quantities.

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[1] Proteins of the Wheat Kernel

[2] The Vegetable Proteins.

When it is thus acted upon, it, is changed to a colorless compound. This is the process of bleaching in flour manufacture. The oil is yellow, due to this coloring matter. It is the coloring matter that is affected in the process of bleaching. Some authorities believe the oxidizing or bleaching agent enters into a chemical combination with the coloring matter, giving a colorless compound. Others are of the opinion that the oxidizing agent reacts on the coloring matter, oxidizing it and thus producing a colorless compound. Most of the fat is located in the germ of the wheat. The outer coat or bran carries the largest amount of coloring matter. Both are found throughout the whole kernel.

The ash is composed chieflynof phosphates such as the potassium and sodium salts. The phosphates to a certain extent are evidently in chemical combination with the other constituents, especially the proteins. This is shown when the gluten is washed out of a flour. No matter how long or carefully it is washed, some mineral matter remains as is shown on ignition. The outside coat of the

kernel or bran is the richest in ash content. The percentage decreases as the center of the seed is reached.

The proteins are the most important constituents of the wheat kernel. They are not sharply defined. Some authors claim a certain number, others a larger or lesser number. Osborne describes five distinct protein substances in the wheat kernel. His classification seems to possess the most reliable foundation. The five proteins are Leucosin, Globulin, Proteose, Gliadin and Glutenin.

Leucosin forms about 0.3 to 0.4 o/o of the wheat kernel. A large proportion of this is in the germ or embryo. It is an albumen because it is soluble in pure water and coagulates on heating.

Globulin also occurs chiefly in the embryo. It is soluble in a weak salt solution. About 0.6 o/o of the wheat kernel is globulin.

The wheat kernel yields a very small amount of proteose when extracted with water. Like leucosin and globulin, it is found chiefly in the embryo.

Gliadin is the most important of all the proteins

because of its unusual physical properties and its effect on the baking value of a flour. It is soluble in the greatest amount in ethyl alcohol 60-70 o/o by volume. The solubility decreases as either the water or alcohol is increased. Gliadin is also soluble in methyl, propyl, and other alcohols. It is somewhat soluble in aniline, pyridine, phenol and glacial acetic acid. It usually is not soluble in these substances alone but with the addition of water, its solution is greatly aided. It is also somewhat soluble in pure water but to a very slight extent. Dilute acids and alkalies likewise dissolve it completely. When such solutions are neutralized, The gliadin is precipitated as a white flocculent powder which collects to a semi-liquid. Other cereals yield a substance which has the same composition, the same solubility, and the same physical properties. Yet the gliadin obtained from the different sources seems to greatly differ. Gliadin has a specific rotation in its solutions. Snyder[1] worked on this trying to show that the per cent of gliadin could be found in a flour by measuring the rotation. The method has been sharply

[1] Det. of Gliadin in Wheat by the Polariscope. Jour. Amer.Chem.Soc., 1904, P.26.

criticized and does not give the results expected yet. The amount of gliadin varies greatly in the different wheats. In the greatest number of cases the gliadin forms from 40 to 60 o/o of the total protein content of the seed and from about 4 to 12 o/o of the total seed.

Glutenin, the second main protein lies next to gliadin in importance. It is quite insoluble in any of the substances that gliadin dissolves in with the exception of the alkalies and acids. It is present in nearly equal amounts as gliadin. On Hydrolysis, according to Osborne, it gives about the same compounds with the exception that it yields glycocoll and lysine. Gliadin does not yield these. In the other hydrolytic compounds it yields a slightly smaller per cent.

Composition of the Proteins.

	Carbon	Hydrogen	Nitrogen	Sulfur	Oxygen.
Leucosin	53.02	6.84	16.80	1.28	22.06
Globulin	51.03	6.85	18.39	.69	23.04
Proteose	49.50	6.80	17.08	1.24	25.50
Gliadin	52.72	6.86	17.66	1.03	21.73
Glutenin	52.34	6.83	17.49	1.08	22.26

There are no large number of analysis of the different constituents of the wheat kernel such as Osborne has made. His results agree fairly well with those that have been made. It would be exceedingly interesting to separate the five proteins that Osborne has obtained from different wheats grown under very different conditions, to see if such relations hold for all of them. Again it would be interesting to repeat his work on the hydrolysis of the proteins.

It may be found that there is only one protein substance in the wheat kernel and the others obtained are only different forms of this one protein just as para formaldehyde is a different form of formaldehyde, yet chemically the same. It will be noticed that the percentage composition of the five proteins differ very little.

There is yet another substance in the wheat kernel, which, until recent years, has been almost wholly ignored although it was known to be present . That is , bacteria, enzymes or ferments. Ford and Gutherie[1]

[1] Anolytic and Proleolytic Ferments of Wheaten Flour Jour. Soc. Chem. Ind. 27, 389(1908)

have analyzed several flours made from different wheats and has shown, entirely from a chemical standpoint, that in many cases the presence and amount of these ferments have a decided influence on the quality of a flour as to its bread making value.

The work on this paper has been confirmed almost wholly to the protein gliadin. It is the easiest to separate from the other constituents in a pure form.

The method of separation is the one that is commonly used. On account of the difficulty of preparing the gliadin free from the coloring matter present, a modification of this method was used. The method was as follows:

Four kilograms of a fairly white flour and fairly high in gliadin was made up to a stiff dough with distilled water at about 30°C. The dough thus obtained was covered with water and allowed to stand for one hour when it was carefully washed in a stream of water. The whole was carefully kneaded with the hands and washed until the wash water showed only traces of starch when the ball of gluten was pressed between the hands. It was then placed in cold

water and allowed to stand over night in a refrigerator when it was again washed. Considerable more starch separated. It was washed until the wash water showed no traces of starch. The process was repeated on standing three hours more. With such a large amount of gluten the repeated washing was necessary.

If the gluten is then allowed to stand at room temperature it begins to ferment. The whole mass fills with bubbles and the gluten becomes very elastic. It can be stretched into long strings by its own weight. The gluten was then extracted with four liters of alcohol of 69 o/o by volume. The gluten was reduced to fairly small pieces after it was placed in the alcohol. Osborne's method of chopping it fine before extraction was tried but it was found that even after the gluten was chopped fine and allowed to stand for a very little time, it adhered together in one mass. The finely divided gluten was allowed to stand with alcohol over night when the supernatant liquid was decanted off. The residue was then placed in a screw press and pressed until all the liquid possible was pressed out. The press was lined with a double thickness

of heavy linen cloth. The residue was again extracted with alcohol as before. The liquid was allowed to settle for twenty four hours and was then decanted. The clear digestion liquids were then all mixed and filtered. It was found that a saturated solution of gliadin in alcohol of 70 o/o by volume filtered with extreme difficulty. When a quantity of the volume of alcohol of the same strength is added, the solution filters much more readily. The process of filtration is also very difficult if there is present any suspended matter. The suspended matter can best be gotten rid of by placing a perforated porcelain plate over the mouth of a large thistle tube and then placing over this two thicknesses of heavy linen. This thistle is fixed up with a suction so that the liquid is filtered through it. The filtrate thus obtained and diluted with 70 o/o alcohol filters fairly easy through a fine filter paper.

The resulting liquid is yellow in color but absolutely clear and free from suspended matter. The yellow coloring matter dissolved in the alcohol causes the color of the solution. It is difficult to prepare gliadin, that is, free from this coloring matter. It could only be done by repeated solution and precipitation.

As it was noted under the properties of the protein gliadin its maximum solubility is in alcohol of 50 to 70 o/o by volume. It was noted also that it is almost insoluble in pure water and in absolute alcohol. The combined filtrates were concentrated to about one quarter of their volume or when the gliadin just began to separate out. The clear liquid was poured in a fine stream with a great deal of stirring into ten liters of absolute alcohol to which one liter of pure ether had been added. The gliadin, being insoluble in absolute alcohol, was precipitated in a very finely divided state. The ether served to keep the coloring matter in solution and keep it from contaminating the gliadin. The jar was covered and allowed to stand until the finely divided gliadin had all settled. The alcohol was poured

off and the precipitated gliadin washed several times with absolute alcohol and then with ether to remove the alcohol. It was then dried in a dessicator over sulphuric acid. If allowed to stand, exposed to the air and dried it again, takes on moisture and becomes black which must be due to oxidation. The gliadin thus obtained is a pure, ash free, snow-white, fraible powder of low sp.gr.

The first work was an attempt to find something about the structure of the gliadin molecule. Fisher has demonstrated that the peptones are built up from amino acids. The gliadin on hydrolysis yields a large per cent of amino acids. It seems as if these amino groups or parts of them might be replaced by an active acid substance so the formation of the protein gliadin was attempted.

The substance being soluble in alcohol, the process was first tried in this solution. Ten grams of gliadin was dissolved in 200 cc. of alcohol with difficulty and ten cubic centimeters of bromine was added. The whole was then boiled on a water bath with a reflux condenser for six hours. This process gave poor results

on account of the bromine acting upon the alcohol. ~~No~~ No constant boiling points were determined because the of the mixtures of alcohol derivatives that came over. A dark reddish looking mass was left as a residue. This residue was quite soluble in chloroform.(gliadin is not) but no crystals were obtained on evaporation. It was found to be impossible to purify it by means of ordinary solvents. No bromine compound was identified.

Owing to the difficulties encountered in in an alcoholic solution, a solution of gliadin in glacial acetic acid was next tried. Ten grams of gliadin were dissolved in 200 cc. of glacial acetic acid. Five cc. of bromine were added which in the cold precipitated the gliadin. Fifty cc. more of glacial acetic was added and the solution warmed and all the gliadin went into solution. The solution was then heated at its boiling point, using a reflux condenser for eighteen hours. At the end of twelve hours there being a loss of bromine, five cc. more were added.

The excess of acid was boiled off and a

substance began to precipitate out.The contents of the distillation were transferred to a beaker and the excess of acetic acid neutralized with sodium hydroxide after dilution. The gliadin was all precipitated. It was colored with bromine but did not differ from the gliadin that was started out with being insoluble in water and soluble in 70 o/o alcohol. From the alcohol solution it was precipitated with an excess of absolute alcohol. It came down as an almost white powder. Bromination might take place in a neutral solution but in an acid solution it was not possible to effect it. This may have been due to the acid and gliadin forming a salt which the bromine will not effect.

It was found that when two electrodes were put in an alcoholic solution of gliadin that the solution conducted electricity. The importance of this can hardly be overestimated. It would indicate that the gliadin dissociated when it goes into solution. The gliadin, however, precipitated on the negative electrode as a white gelatinous mass. Hydrogen was given off from the other electrode. If the current was long enough nearly all the

gliadin could be precipitated from the solution. One very interesting thing was noticed when flour is extracted with neutral alcohol directly and then the current passed through precipitation of the gliadin does not take place until a certain amount of the alcohol is changed over into acetaldehyde. If the gliadin has been prepared pure and then dissolved, the precipitation takes place as soon as the current begins to flow through the solution. In order to be sure that the large conductivity obtained was not due to impurities, the alcohol was distilled over potassium hydroxide and the water was redistilled. This solution was found also to conduct. It was thought that this substance that was precipitated on the electrode might possibly differ chemically from the pure gliadin although it had the same physical properties. A combustion for nitrogen was run on a sample of the substance thus obtained. The substance was found to contain 17.86 o/o of nitrogen which corresponds within the limits of experimental error, to the total nitrogen found in gliadin. On account of the lack of time, the measurements of the conductivity of gliadin solutions

were not made. It is known that the gliadin and a base unite because it takes considerable more potassium hydroxide to make a gliadin solution alkaline than that calculated for the acid present. This would indicate that the salt of gliadin was formed. In the near future the writer hopes to measure the conductivity of the different solutions of gliadin such as alcohol, acetic acid, potassium hydroxide and etc.

In the purification of the alcohol from the alcoholic extracts which was done over potassium hydroxide, it was found that when the solution reached a certain concentration, an oily substance began to separate out. This, of course, was very strongly baric in character due to the potassium hydroxide. It was of a brown color and a fairly high sp.gr. Purification of this substance was attempted. It was found to be quite soluble in water and in alcohol and ether. In the course of the purification, seeing the substance separated from about a 50 o/o solution of ethyl alcohol, this was tried as a purifying agent. It was soluble in this solution. Methyl alcohol was next tried but it was soluble in it. It was noticed

that the substance was soluble in methyl alcohol of 50-70 o/o by volume but if absolute methyl alcohol was added in sufficient quantity, a white flaky substance settled out. This substance was taken out, washed with absolute methyl alcohol until all the KOH was removed. It was then washed with anhydrous ether and dried in a desiccator. Before drying, the substance was very volumenous but after drying, it was reduced to a very small amount. The amount of substance was too small to work with so the preparation of more substance was attempted.

Am alcoholic solution of gliadin was treated with KOH and warmed. A strong odor of ammonia was given off. Osbourne says that about a third of the total nitrogen is given off when wheat proteins are treated with strong alkalies. The amount given off was found out
Two determinations were made.

0.5 gram of gliadin was put in a Kjeldahl flask and treated with 250 cc. of water in which 20 grms. of sodium hydroxide were dissolved. Sodium hydroxide was used because potassium hydroxide foamed so when heated.

The distillate was treated with n/10 H_2SO_4.

.5 gram used 17.5 cc. n/10 H_2SO_4 required

.5 '' '' 17.4 '' n/10 H_2SO_4 ''

$$\frac{.0014 \times 17.5}{.5} = .0490 = 4.90\ o/o$$

$$\frac{.0014 \times 17.4}{.5} = .0480 = 4.85\ o/o$$

After the contents of the beaker had ceased to of ammonia on prolonged boiling, it was poured into a large volume of absolute methyl alcohol, When it precipitated out as before. This substance differed from gliadin in that it was quite soluble in distilled water. From this it was thought that it might differ from gliadin chemically.

Several nitrogen determinations were made on samples of the substance.

Amt. used	cc n/10 H_2SO_4	Per cent Nitrogen.
1.14 grms.	63.5	7.76

Two more determinations were made and the results were 7.50 o/o and 7.87 o/o. Determinations made on another preparation gave 8.20 and 8.56 o/o respectively. Determinations on further preparations were not made.

From the above results, it will be noted that the amount of nitrogen in the compound is just about half that found in gliadin itself. According to Fisher's formula for a polypeptid, $CH_3 \cdot CHNH_2 \cdot CO \cdot NH \cdot CH_2 \cdot CO \cdot NH \cdot CH_2COOH$. It is generally thought that the proteins are a higher form of them. It may be possible that the protein molecule has been split in two. It may be that due to its having both acid and basic properties, the basic KOH combined with the acid part of the molecule to form a salt and the rest of the molecule combined with the OH group to form another compound.

A water solution of this compound was used to get a double compound with platinic chloride. The solution was evaporated but no results were obtained.

Conclusions.

It is concluded that

1) The gliadin molecule is quite stable because it resisted the action of a strong agent like bromine.
2) That bromine cannot be made to enter into the molecule by direct means.
3) That the protein substance gliadin is dissociated in solution.
4) That the molecule is broken down by means of a strong potash solution. That the substance formed is a new substance because it contains about half as much nitrogen as the original gliadin.